AWARD NUMBER: W81XWH-10-1-0015

TITLE: tRNAs as therapeutic agents of breast cancer

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REPORT DATE: July 2011

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
1 July 2011	Annual	1 Jul 2010 – 30 Jun 2011

4. TITLE AND SUBTITLE

tRNAs as therapeutic agents of breast cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-1-0015

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Chicago
Chicago, IL 60637

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Breast cancer refers to a variety of types that each derive from distinct causes and merit different treatments. It is critical to apply a variety of treatment options that target a wide range of cellular mechanisms and targets, including those that are previously unexplored to enable new routes of therapy. Our proposal explores a new and previously under-appreciated avenue which is to use specifically engineered tRNAs that are always attached with an incorrect amino acid in a human cell. This mismatch of amino acid identity to the coding capacity of the tRNA results in increased production of mutated proteins which are misfolded, leading to over-simulation of an intrinsic cellular process that causes cell death. We are studying the effect of these engineered, mischarged tRNAs on breast cancer cells. Ultimately, we aim to demonstrate the suitability of these engineered RNAs as a new class of agents for the treatment of breast tumors.

15. SUBJECT TERMS
tRNA, therapy, delivery, breast cancer

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	7	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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INTRODUCTION:

Transfer RNAs (tRNA) are small non-coding RNAs that read the genetic codes in protein synthesis. It is essential for the proliferation, fitness and adaptation of the cell that each tRNA is aminoacylated (charged) with its designated amino acid. The utilization of mischarged tRNAs (i.e. tRNAs with incompatibly charged amino acid and decoding capacity) leads to the synthesis of mutated proteins that can fold incorrectly. Accumulation of misfolded proteins in the cell activates an integrated cellular mechanism, the Unfolded Protein Response which dictates cell fate in response to the amount of misfolded proteins. High accumulation of misfolded proteins derived from the cellular presence of mischarged tRNAs can therefore induce apoptosis of the cell (1). We aim to engineer tRNAs that are always mischarged in a human cell and study their effects on breast tumor cell physiology and cell death. Protein demand in rapidly proliferating cells is extremely high and small defects during cellular protein synthesis caused by the presence of such tRNAs can have a strong impact on both tumor invasiveness and survival. Ultimately, we aim to demonstrate that these mischarged tRNAs can be developed as a novel class of RNA-based agents to treat breast tumors.

BODY:

Task 1: Confirm that mischarged-tRNA (termed "killer-tRNA" for its ability to kill cancer cells) works in two breast cancer cell lines. → **Completed successfully.**

We analyzed the impact on two model strains: MDA-MB231 (Her2-) and BR474 (Her2+). Killer-tRNA blocks overall translation at 24h and kill cells at 48h in a dose dependant fashion as shown by lipofection of different amounts of killer-tRNA (Figure 1). Wild-type $tRNA^{Ser}$ is used here as a control.

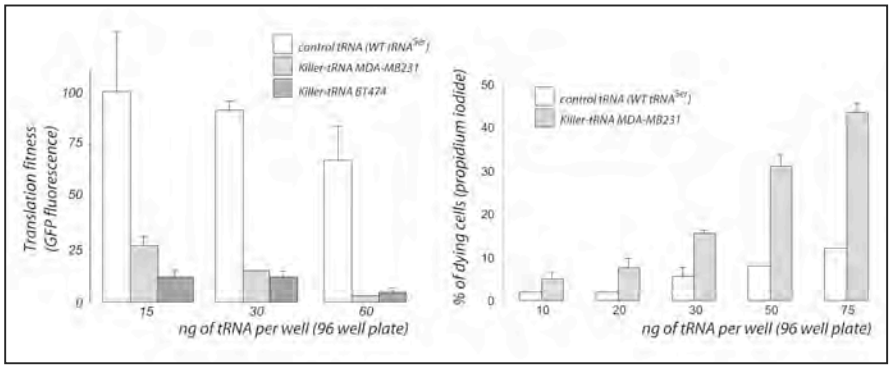

Figure 1. Effect of killer tRNA on breast cancer cell lines. (Left) Translation fitness. (Right) Percent dying cells. The wild-type tRNA control is shown as open bars and the breast cancer lines are in shaded bars.

Killer-tRNA also drastically reduces growth rate of both breast cancer cell lines (Figure 2). The amounts of killer-tRNA lipofected is per well of 96-well plate.

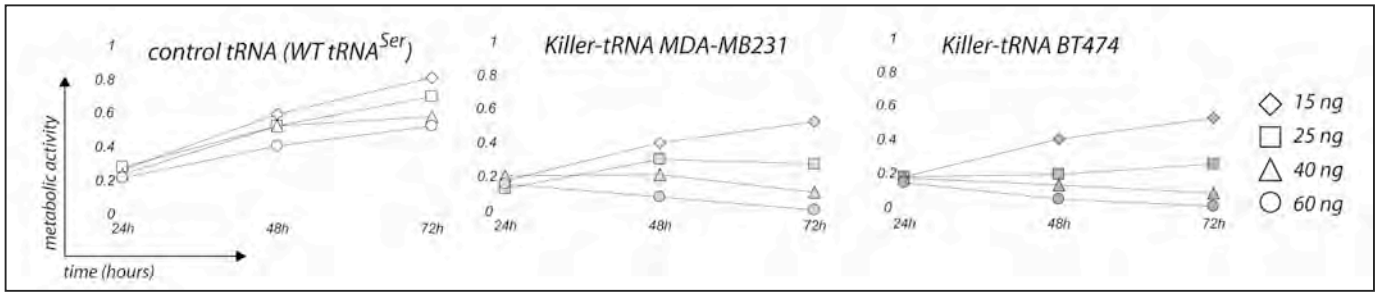

*Figure 2. Strong effect of killer-tRNA on growth rate*s. (Left) Control wild-type tRNA; (middle) MDA-MB-231 (Her2-); (right) BT474 (Her2+).

Task 2: Examine the effect of mischarged tRNA (killer-tRNA) in mice bearing xenograft tumors. **→ In progress.**

We first established the protocol for doing the mouse work with killer tRNA. In this case, the amount of killer-tRNA for lipofection and timing for cell collection are experimentally parameters that had to be optimized. Our optimization shows that we can transfect 500,000 cells with 800 ng of killer-tRNA per well during 4 hours, collect cells at 8 hours to be immediately injected in mice (Figure 3). Cells treated with killer-tRNA are already sick as shown by their reduced metabolic activity but not yet dead. Onset of cell death is expected to occur around 36 hours for this dosage if cells follow similar trend *in vivo* as in cell culture.

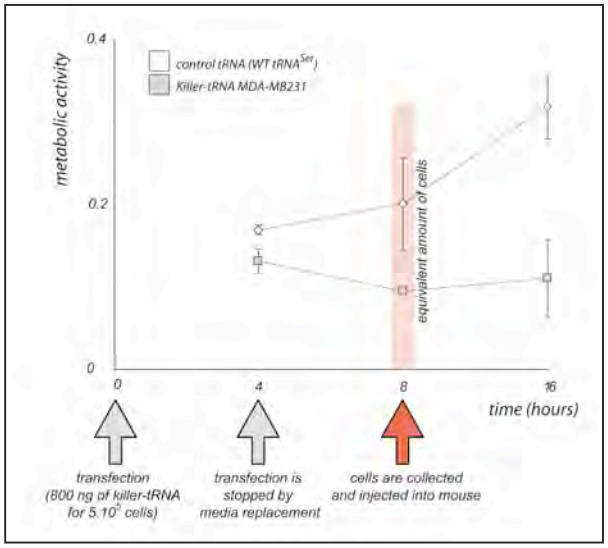

Figure 3. Optimization of killer tRNA amount and timing before mouse injection. For MDA-MB-231, killer and wild-type control tRNA are transfected from 0-4h, followed by removal of the transfection agent. Cells are incubated for another 4h before collection. The collected cells are immediately injected in mice.

We plan to inject killer and the control wild-type tRNA into 10 mice each in July 2011.

Task 3: Explore delivery methods of mischarged tRNA (killer-tRNA) to mouse tumors. **→ In progress.**

a. Design tRNA scaffolds that enable the expression of the conditional reporter.

In comparison with standard lipofection, the efficiency of nanoparticle-mediated delivery is expected to be in the low to mid range. The onset of the apoptotic response associated with killer tRNA is dose dependant. Therefore, rather than waiting for complex phenotypical changes in the targeted cells we proposed to design a fluorescent screening procedure to monitor the internalization and the usage in translation of tRNA based drugs. This procedure includes a set of three similar molecules: killer-tRNA, wild-type tRNA, and suppressor tRNA (Figure 4). They share identical tRNA^{Ser} bodies (96 % of the

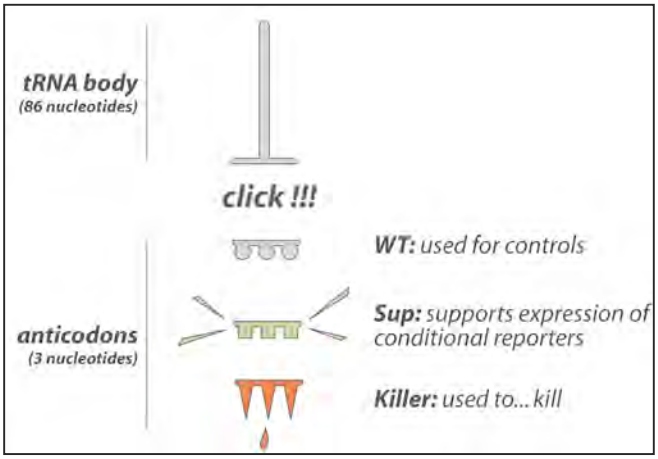

Figure 4. Scaffold of three tRNAs used in the delivery study. These tRNA differ only in their anticodon sequence. Wild-type tRNA reads codon for serine, Suppressor (Sup) tRNA for amber stop, and killer tRNA for isoleucine.

molecule) but display different anticodons. Suppressor tRNA was engineered to base pair with amber stop codons and support translation of reporter proteins harboring amber non-sense mutations in their open reading frame. Delivery strategies effective for the suppressor tRNA will be directly applicable to the killer-tRNA, its toxic mimic.

b. Design and test of a tandem meGFP (constitutive) and mCherry (conditional) reporter.

These two tandem protein reporters have distinct excitation and emission spectrums allowing simultaneous study by flow cytometry and fluorescent microscopy. The gene encoding the fusion protein harbors a non-sense mutation in the linker between meGFP (green) and mCherry (red). In the absence of suppressor tRNA, only meGFP could be expressed (tested here with Hela cells for transfection convenience, Figure 5). Co-transfection of suppressor tRNA allowed the read-through of the non-sense mutation and supported the expression of the full-length dual reporter. We thus established the basis for a simple screening procedure to monitor tRNA incorporation into cells based on fluorescence.

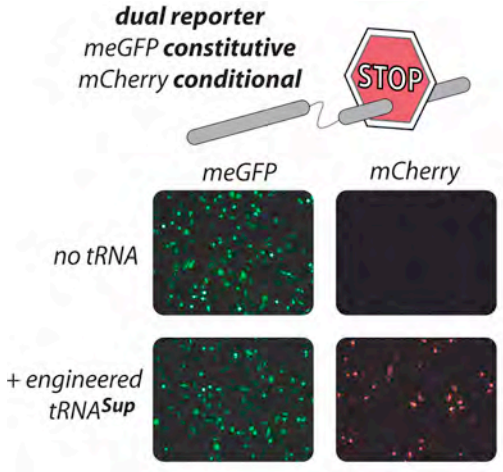

Figure 5. Design and test of a dual reporter to monitor delivery efficiency. The meGFP signal indicates cells that contain this dual reporter, whereas mCherry signal is only present when cells are successfully transfected with tRNA.

c. Nanoparticle delivery study.

We attempted to deliver our set of three tRNAs using nanoparticles formulated by LNK Chemsolutions (Lincoln, NE). We show that the nanoparticles we use in our study are able to deliver a fluorescent molecule in cultured cells (Figure 6). We are currently optimizing solution conditions to package sufficient amount of tRNA in these nanoparticles for our proposed studies.

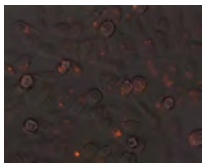

Figure 6. Delivery of a fluorescent dye using nanoparticles. Cells with nanoparticle-delivered rhodamine show red fluorescence.

KEY RESEARCH ACCOMPLISHMENTS:

- Demonstrated that killer tRNA is effective for a wide range of cancer cells.
- Determined optimal conditions for killer tRNA study in animals.
- Constructed dual reporter system for efficient monitoring of tRNA delivery.

REPORTABLE OUTCOMES:

- Poster presentation at the Era of Hope meeting, August 2011.
- Constructs of killer, suppressor and wild-type tRNAs.
- Construct of dual reporter plasmid.

CONCLUSION:

Our results show for the first time that killer tRNAs are effective agents to kill breast cancer cells. We further show that killer tRNA acts to affect cellular protein synthesis activity in breast cancer cells. In the second year of our study, we will investigate the effect of killer tRNA in mice using our optimized condition for collecting cells that are transfected with killer tRNA. Furthermore, we will optimize nanoparticle packaging conditions to enable direct delivery of killer tRNAs to specific cell types.

RNA interference is becoming an important new class of drug and considerable efforts have been made to develop methods to shuttle small interfering RNA (siRNA) molecules into target cells. The primary mode of action of siRNA based approaches is to target and degrade specific messenger RNAs. Our tRNA-based therapeutic approach is based on an entirely different mechanism utilizing a completely different pathway compared to siRNA therapeutics. We envision that our approach will complement siRNA approaches and offer a different route of treatment for breast cancer.

REFERENCES:

(1) Geslain, R. et al., *Nucleic Acids Research* 38, e30, 1-11 (2010).